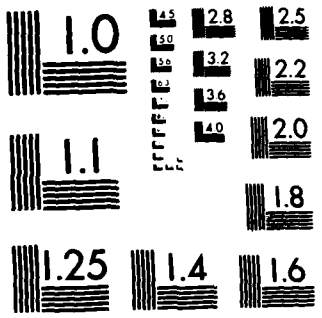
AD-A140 131 MECHANISMS OF RETINAL DAMAGE FROM CHRONIC LASER RADIATION; THRESHOLDS AND MECHANISMS(U) LOUISVILLE UNIV KY DEPT OF OPHTHALMOLOGY T LAWWILL ET AL. JUN 80 1/1

UNCLASSIFIED DAMD17-74-C-4026 F/G 6/18 NL

END
DATE FILMED
5-84
DTIC



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

AD _____

MECHANISMS OF RETINAL DAMAGE FROM CHRONIC LASER RADIATION
Thresholds and Mechanisms

Annual Report

by

Theodore Lawwill, M.D.

Robert S. Crockett, Ph.D.

Glenna Currier, B.S.

June 1980

Supported by

US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-74-C-4026

Department of Ophthalmology
University of Louisville
School of Medicine
Louisville, Kentucky 40202

This document has been approved for public release; distribution unlimited.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A140 137	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) MECHANISMS OF RETINAL DAMAGE FROM CHRONIC LASER RADIATION - Thresholds and Mechanisms		5. TYPE OF REPORT & PERIOD COVERED Annual - 1 November 1976 - 30 June 1980
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Theodore Lawwill, M.D. Robert S. Crockett, Ph.D. Glenna Currier, B.S.		8. CONTRACT OR GRANT NUMBER(s) DAMD17-74-C-4026
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Ophthalmology, University of Louisville, School of Medicine Louisville, Kentucky 40202		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62773A.3E162773A819.00.068
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick, Frederick, Maryland 21701		12. REPORT DATE June 1980
		13. NUMBER OF PAGES 42
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

This document has been approved for public release and sale; its distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ABSTRACT

The effect upon the retina of exposure to large fields of bright visible light has been evaluated. The thresholds for permanent retinal damage for 4,1, and 0.25 hour exposures in rhesus or cynomolgus monkeys have been established for laser lines of 514.5 nm, 488 nm, 457.9 nm, and 590 nm. In addition, the effect of distributing the 4 hour exposures in one hour doses separated by intervals of one day or one week has been studied. The damage has been evaluated by ophthalmoscopy, electroretinography, fluorescein angiography and light and electron microscopy. The shortest wavelength light (457.9 nm) is more effective in causing damage, particularly histological damage, which is spread throughout the fundus and throughout the retinal layers. There appears to be more than one mechanism for retinal damage in chronic light exposure, and at least one mechanism is not dependent solely upon the visual pigment and the pigment epithelium. The results from our studies suggest that with relatively low intensity, long duration exposures that there is a lack of reciprocity between exposure duration and irradiance level. There appears to be a saturation effect in which during exposures of increasing duration little additional damage occurs. A similar effect occurs when the interval between exposures is increased beyond a critical duration, i.e. the threshold for damage from 1 hour exposures separated by 7 day intervals is not appreciably different from a single 1 hour exposure, whereas with shorter intevals between exposures the effect is additive. Thresholds of permanent damage appear to be within one or two log units of light levels encountered in the normal visual environment.

FOREWARD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

TABLE OF CONTENTS

PURPOSE OF PROJECT

The thrust of this project is to define the mechanism by which light at intensities insufficient to cause thermal effects induces pathological changes in the retina. Stated in its strongest form our aim is to identify the biochemical entities absorbing photic energy and to define the path by which this energy is transduced and leads to alteration in structure and function.

INTRODUCTION

Our work in chronic light damage began in 1967, shortly after Noell's (1966) report on retinal damage in rats. We have established the permanent damage threshold for white light and several lines of the argon laser in rabbits (Lawwill, 1973a) and monkeys (Lawwill, 1972, 1973b, 1976). The threshold retinal irradiance for damage in these two diurnal animals for four hour exposures to white light is at least three log units higher than that in the rat (Lawwill, 1972, 1973b). Noell (1971) has reported that in the rat the damage shows a spectral sensitivity similar to visual sensitivity. The spectral sensitivity of damage in the rat is different from that which we have found (Lawwill, 1972, 1973b) and from that which Ham (1976) has reported in monkeys.

In our experimental model, we are exposing a large area of the posterior pole to a relatively even illumination of moderately bright light for a period of hours. We are not dealing with the thermal damage caused by small spot laser burns and photocoagulation. The level of light we use is unlikely to raise the temperature of the retina even one degree centigrade (Clarke and Geeraets, 1969).

We find not only that the threshold is lower with blue light, but that the morphological changes may be slightly different from those previously described for white light and 514.5 nm laser light. Primary damage occurs in retinal layers which we think of as relatively transparent to the damaging wavelength. We find that the variability throughout a

single fundus is large, both as to which layer is affected and as to whether there is damage. The location and degree of damage may be very different in physically adjacent areas of the fundus.

METHODS

Exposure Procedure

The experimental animals are female cynomolgus monkeys weighing between 3 and 4 kg. Prior to exposure, the animal is given 0.12 mg of atropine sulfate and 8 mg of phencyclidine HCl intramuscularly. When the monkey is sedate, it is placed in a primate chair. Initiation of anesthesia is by means of a 20mg/kg intravenous dose of sodium pentobarbital. A constant intravenous infusion of sodium pentobarbital (0.22 mg/min., 2.84 mg/ml) is begun and is continued throughout the four hour exposure. The eye to be exposed is dilated with one drop each of atropine 1%, tropicamide 1%, and phenylephrine 10%. The light is presented to the eye in Maxwellian view. The eye lid is held open by a Burian Allen contact lens electrode. This protects the cornea and keeps it moist. The alignment is maintained by the experimenter's sighting through a beamsplitter to allow direct visualization of the exposed fundus. The intensity of exposure is constantly monitored via a beam splitter in the light path reflecting on an Eppley thermopile. Exposures are for a period of from 15 minutes to four hours with a constant intensity light source covering 50.1 degrees solid angle (0.88 cm^2 of retina). The intensity of the beam is measured with a Gamma Scientific Model 2020a spectroradiometer calibrated with a standard of spectral irradiance traceable to the National Bureau of Standards. The homogeneity of the field is checked with a small (2.5 mm diameter) cosine receptor by measuring the intensity at the center and edge of the field. The uniformity is maintained within 50%.

The retinal area exposed is calculated both from the angle

of convergence of the incident beam and from direct measurement of the chord of the exposed section and the diameter of the eye using a freshly enucleated monkey eye. The area value used in calculating the irradiance is taken from the direct measurement. There is about a 20% difference between the two results.

We evaluate retinal damage induced by light by four measures: Electroretinogram, (dark adapted, pan-retinal flash and light adapted, localized pattern), funduscopy and fluorescein angiography, light and electron microscopy. Our methods of evaluation have been reported (Lawwill, 1973b, 1972, 1976, and 1977a,b).

Flash ERG Procedure

The flash ERG is recorded prior to exposure at least twice a week until a stable amplitude is achieved. At 24, 72, and 144 hours after exposure, and two or three times per week thereafter, the ERG is recorded binocularly. In brief, the animal is tranquilized, following pretreatment with atropine sulfate I.M. (0.012mg/kg) with 8 mg phencyclidine HCl, I.M., and the pupils are dilated with tropicamide 1% and phenylephrine 10%. Modified Burian-Allen type monkey contact lens electrodes are inserted, and the animal is preadapted in a 1370 cd/m^2 white ganzfeld hemisphere. The flash stimulus is provided by a Grass PS2 photostimulator with the intensity set at sixteen. The flash lamp of the Grass instrument is placed inside the hemisphere to provide a ganzfeld type stimulus. ERG's are recorded, beginning three minutes after completion of two minutes light adaptation and then every three minutes

for twenty-seven minutes. Evaluation of the functional damage after exposure is made on the basis of the decrease in amplitudes of the a & b waves and the persistence of this decrease after exposure. The response of the opposite eye serves as a control during this period. The damage is graded on a scale from 0 to 4+. In all cases, the quantification of damage is carried out in a blind manner.

Spectral Bar ERG and acuity function

A three channel optical apparatus (1) images a pattern stimulus on the fundus, (2) selectively illuminates the optic disc, and (3) allows the experimenter to observe the position of the pattern and the disc directly. Through temporal modulation of a grating pattern, the electroretinogram and visual evoked responses are elicited.

Cynomolgus monkeys (Maccaca Fasciculata) weighing 3 to 4 kilograms are injected intramuscularly with phencyclidine HCl mg/Kg. When the animal is sedate, the cornea is anesthetized with tetracaine and the pupil is dilated with phenylephrine (10%) and cyclogyl (1%). With the aid of a laryngoscope, the pharynx and larynx are anesthetized with a benzocaine (14%) spray. Three minutes later, an endotracheal tube with pressure cuff is inserted. The monkey is secured in restraint chair with velcro torso strap, bite bar and head rest. A butterfly infusion set is inserted inravenously and a saline drip begun to facilitate later administration of drugs. A contact lens electrode is installed in the eye to be tested, and needle electrodes are inserted into the scalp for recording of the visual evoked response. After the animal is positioned in

front of the optical apparatus, the saline drip is discontinued and a priming dose of 5 mg/kg of gallamine triethiodide is administered intravenously to induce paralysis. A solution of gallamine in saline (2.8 mg/ml) is administered intravenously with a continuous infusion pump at the rate of 0.051 ml/min. for 120 minutes. The dose of gallamine is selected to eliminate eye movement. When voluntary respiration has ceased, the endotracheal tube is connected to a mechanical respirator. End tidal % CO_2 is monitored and maintained at 4%. The electrocardiogram is monitored with a lead II configuration. A heating pad is wrapped around the animal to maintain body temperature. Two hours following induction of paralysis the infusion of gallamine is terminated and atropine sulphate 1.3 mg/kg is injected intravenously. Five minutes later neostigmine methylsulfate 0.3mg/kg is slowly infused over thirty minutes to reverse blockade of the neuro-muscular junction. When the animal begins voluntary respiration, the respirator is disconnected. Local anesthesia is again applied to the laryngeal cavity and the tube is removed. The animal is observed in the laboratory for at least 30 minutes following recovery from respiratory paralysis. Once returned to its quarters, the animal is periodically observed until she can move about the cage normally.

The necessity of using a neuro-muscular blocking agent is based upon several considerations. The animal must be restrained, contact lens electrodes inserted in each eye, and eye movements eliminated. Therefore, some sedation, anesthetization or paralysis is required. Initially we used

phencyclidine alone. However, this induced a vertical nystagmus which made recording of pattern evoked responses and ERG impossible. The inhalation anesthetic, methoxy-flourane, was then tried. This agent was not suitable for a two hour procedure due to its nephrotoxicity; neither was its effect on the responses measured known. A review of the literature revealed that pentobarbitol altered components of the ERG and, depending upon the level of anesthesia induced, the VER would be reduced in amplitude. Nitrous oxide was not used due to the difficulty of maintaining adequate oxygenation without blood gas measuring equipment.

After evaluation of our protocol, we concluded that the pain or discomfort of our procedures did not require general anesthesia. Contact lens electrodes are routinely installed in humans with only local anesthetic in the eye. Tracheal intubation and extubation are accomplished with only local anesthesia in human patients for diagnostic procedures. We further concluded that the use of a neuro-muscular blocking agent was in order with the following stipulations: (1) preparation of the animal should be accomplished while in a "dissocaited" state induced by phencyclidine, (2) laryngoscopy, intubation, and extubation should be performed after application of a local anesthetic to the pharyngeal and laryngeal cavities, (3) vital life signs, including EKG and end tidal CO_2 concentration should be continuously monitored, (4) the duration of blockade should not exceed 2 hours, (5) reversal of blockade should be produced by the administration of neostigmine following injection of atropine, and (6) the interval between test sessions should exceed three days.

During the early period, as parameters of the technique were being established, we lost three monkeys. The deaths of two were attributable directly to the procedure. However, after over 60 sessions we have encountered no untoward consequences. The entire procedure is carried out by one person. It is not unusual to observe the monkey eating dry food biscuits and fruit within 30 minutes following a session.

It is our opinion that the procedure is one which meets scientific standards in allowing the recording of responses not confounded by drug effects while meeting ethical standards in the humane treatment of animals.

Funduscopic and angiographic procedures

Ophthalmoscopy and fundus photography is performed regularly before and after exposure with the indirect ophthalmoscope and Zeiss fundus camera. A change is graded on a scale of 0 to 4+, without the grader having knowledge of the exposure level. The slightest question of edema or pigmentary change is graded as $\pm$. A definite change in appearance of the fundus no matter how transient, is graded 1+. A 4+ grading is assigned when there is extensive damage to the retina and pigment epithelium which persists indefinitely, showing apparently stable findings after exposure.

Fluorescein angiography is performed prior to exposure on each eye and again after exposure, providing good photographic documentation of changes, particularly those changes in the pigment epithelium.

Histological procedure

The procedure for histological examination is begun by anesthetization of the light adapted animal with Nembutal. The preanesthetic medication is atropine and phencyclidine.

During enucleation the rectus muscles are cut 1 cm from their insertion on the eye. Immediately after enucleation alligator clips are fastened onto the four rectus muscles and the whole eye is placed in a plexiglas open cyliner (3/4 inch inside diameter and 1 inch high) that has been grooved to hold the alligator clips. This cylinder holds the eye suspended and does not allow the posterior pole to collapse when ocular pressure is reduced. The cylinder and globe are then immersed in a bowl of fixative. This laboratory uses 3% Glutaraldehyde in a .1 M phosphate buffer for 1-24 hours at room temperature as the initial fixative. The cornea is immediately removed with a trephine, and four cuts are made radially into the sclera between the rectus muscles. The zonules are cut and the lens removed. A 5cc syringe with a 20 gauge needle is used to gently flush the eye with fixative, and the vitreous is cut out with scissors as it is forced to the anterior opening. Finally, the iris is cut away. With practice this procedure takes less than 3 minutes after enucleation. Using this technique, the retina does not detach even with severely damaged eyes. This laboratory has processed 124 consecutive eyes without detachment and has been able to produce repeatable histological fixation.

The eye is cut into triangles, placed in tubes and labeled

The tissue is placed in a phosphate buffered wash to remove the glutaraldehyde, post-fixed for one hour in 1% OsO_4 at 4^oC, dehydrated with alcohols and then infiltrated with plastic embedding media. At this time each triangle is cut into six smaller pieces, oriented in flat embedding molds (Ladd Scientific), and numbered according to a numbered grid map of the retina. The blocks are hardened overnight at 65^oC. One micron thick sections are cut on a LKB Ultramicrotome, placed on a glass microscope slide and stained with 1% toluidine Blue in a phosphate buffer. After rinsing and drying, the slide is coverslipped and viewed under oil with a Zeiss Photo-microscope. The combination of the thin plastic sections and the use of the oil immersion lens, allows structures to be viewed with the light microscope that cannot be seen with the conventional Hematoxylin-Eosin stained paraffin tissue sections.

Histological evaluation

Different cells have different functions and metabolism, sometimes because of their shape or size and other times because of their location or the needs of the organism. Cells react differently even though the stimulus or trauma is the same for all. This evaluation must be carried out individually for cell groups. For this purpose, the retina is divided into 10 specific la[illegible]s and the normal histology is determined for each layer. [illegible] deviations from normal for each layer are listed in appr[illegible] order of severity.

A grading system has been developed in this laboratory for other methods of examination including electroretinography and indirect ophthalmoscopy. Each method has been assigned

a zero to four grading scale, and this range has been very effective for data handling. In order to apply this already workable system and to allow comparable data to be entered into the computer, the 0-4 grading has also been adopted for histological evaluation. This system is similar to the cytological grading systems used for cancer detection and like these schemes can be adapted to processing and grading of similar material by different individuals.

The grading system must be established in each laboratory by describing the range of normal for that laboratory. After this task is accomplished, the affected retina can be graded by using a criteria sheet (Figure 1).

On the criteria sheet, Animal # refers to one animal, Specimen # refers to one eye of that animal and the Grid # relates to a specific area in that eye. Each slide from each area of the eye has its own criteria sheet. The code is the grade of 0-4 for each layer. Most of the terms are defined in Stedman's Medical Dictionary. Terms that need clarification are listed and defined below.

edema - Extracellular swelling,

ghosting - fading of nucleus, Figures

macrophage - phagocytic cell, origin not specified.

pigment shift - melanin pigment has moved away from its position to areas of the RPE cells where is not usually found.

pigment balling - pigment in the RPE is clumped into balls rather than elongated cigar shaped bodies.

hyperplasia - a large increase in cell numbers of that

Spec. #		Grid #			Animal #		
		Code	– 0–1	– 2	3	– 4	Remarks
1	PE		O vacuolization O edema O hyperchromasia O ghosting	O phagocytes O pigment shift O ↓ in lysosomes	O pigment↓ O pigment balling O absence of pigment	O absence O phagocytes↓ O hyperplasia O dead cells	
2	OS		O disorientation O slight swelling	O phagocytes O mild swelling	O extreme swelling O bizarre forms	O absence O phagocytes↓ O dead cells	
3	IS		R C O O vacuolization O O edema	R C O O phagocytes O O slight swelling	R C O O extreme swelling	R C O O absence O O phagocytes↓ O O dead cells	
4	ONL		R C O O vacuolization O O edema O O hyperchromasia	R C O O phagocytes O O ghosting O O nuc. halo O O chrom. clumping	R C O O lg. nuc. halo O O ↓ cell numbers	R C O O absence O O phagocytes↓ O O pycnosis O O dead cells	
5	OPL		O vacuolization O edema	O phagocytes O slight swelling	O extreme swelling O cell numbers↓	O phagocytes↓ O dead cells	
6	INL		O vacuolization O edema O hyperchromasia O *ghosting*	O phagocytes O nuc. halo O chrom. clumping	O lg. nuc. halo O ↓ cell numbers	O absence O phagocytes↓ O pycnosis O *dead cells*	
7	IPL		O vacuolization O edema	O phagocytes O slight swelling	O extreme swelling	O absence O phagocytes↓ O dead cells	
8	GCL		O vacuolization O edema O hyperchromasia	O phagocytes O ghosting O chrom. clumping O slight swelling	O extreme swelling	O phagocytes↓ O pycnosis O dead cells	
9	Chor.		O edema, slight O ↑ inflammatory cells O ↓ *size Bruchs Memb.*		O vessel occlusion O ↑↑ inflammatory cells O *breaks in Bruchs Memb.*		
10	Ves.		O thickening of walls O ↑ inflammatory cells		O occlusion		

Figure 1. Criteria sheet for scoring type and degree of histopathology.

one layer has occurred.

nuclear halo - there is swelling of the nuclear membrane away from nucleus, giving the appearance on the light microscope of a clear ring around the nucleus. This could also be associated with a condensation of chromatin and a decrease in size of the nucleus.

As the slides are examined, the layers are viewed in the order listed on the criteria sheet; and the circles are checked if any of the cellular changes are found in that layer. A layer that has no circles checked is considered normal and given a 0 grade. This is only a qualitative evaluation, to be more quantatitative "plus" signs may be added after the description to denote a large amount (example) 10-15 vs 30-40 dead cells. 1 or 2 dead cells must be noted but does not indicate a four grade for that layer. "Few" written after the word dead cells indicate less than 5. The remarks column can be used to record exceptions or pathology not listed.

Tissue culture procedure

The RPE cells are extracted and cultured according to a method modified from Gonasun and Potts (1974). Either the primary cultures or first-generation subcultures are used for the experiments. Some melanin is retained in such young cultures. The cells are irradiated in a double-walled plexiglas box. Temperature is regulated to $37 \pm 1^{\circ}C$ by circulating water from a constant temperature bath around the inner walls. Moist 5% CO_2-air mixture is passed continuously into the inner compartment throughout the experiment to mimic the environment of the regular culture chamber.

The light source is a 3W argon ion laser with a Littrow prism wavelength separator. The irradiance is calibrated against a standard source with a spectroradiometer (model numbers 220-9A and 2020A, Gamma Scientific Inc., San Diego, California). The laser beam is dispersed with a lens so that the illuminated area at the monolayer of cells exhibits a Gaussian profile approximately 2 cm in diameter. This was checked with a photodetector covered with a 0.75 mm pinhole moved across the 2 cm circle of illumination. After correcting for surface reflection and culture medium absorption, the apparent absorption by the cell layer was found to be around 10 percent at the wavelengths studied in this report. Since such measurements include a significant contribution from cell scattering, the true absorption by the cells might be appreciably lower. After each experiment, the samples are returned to the incubator and allowed to continue to grow for 24 hours. With the aid of visible markers on the petri dish, one can focus on and monitor changes in the same selected area of the sample. The cells are fixed with 3% glutaraldehyde and stained with 1% toulidine blue for brigh-field microscopy.

Fundus reflectomery

The fundus reflectometer is modeled after one described by Ripps and Snapper (1974). Our modification consists of replacing their bleaching optical channel with our laser exposure system to facilitate rapid alternation between measuring reflectance spectra and exposing the monkey retina in the usual manner. Another change in our apparatus is the implementation of photon counting, a digital method, instead of analog

voltage measurement from the detector. This approach increases the sensitivity, resolution, and dynamic range of the reflectometer.

Monkeys are prepared as described for laser exposure and placed in the exposure apparatus. A reflectance spectrum is recorded before, at varying intervals during, and at varying intervals after exposure. Because the animal is not moved and because the fundus can be observed directly by the experimenter, we are assured of testing only the retinal area with the exposure field.

By comparing difference spectra between the pre-exposure spectrum and those at post-exposure intervals with difference spectra following non-damaging flash bleaches, we will be able to detect abnormalities in the regeneration kinetics of rhodopsins in the laser exposed retina. In addition, this technique may allow us to detect the presence of a novel chromophore resulting from exposure whose absorbance spectrum matches the action spectrum of damage. Our ability to accomplish this depends upon the extinction coefficient of the chromophore, its concentration, its stability, as well as the sensitivity of our measuring technique.

Results

Initially our subject was the rhesus monkey. Approximately 100 eyes were exposed to delineate the action spectrum of damage for a 4 hour continuous exposure. In 1978 due to the increased cost of this specimen and more importantly, the lack of availability, we elected to change to the cynomolgus monkey. This change necessitated redefining the model. This has been accomplished. Table I lists physical measurements made on the globe size of the two specimens. These data were used to calculate the retinal irradiance of exposure. When damage thresholds for the two specimens are based upon retinal irradiance (uncorrected for ocular media absorption) the values obtained are the same.

The largest portion of our effort has been directed to determining the action spectrum of damage as a function of several parameters. These factors are summarized in Table IIa and IIb. Our goal in this series of experiments is to (1) determine the degree of reciprocity between exposure duration and irradiance and (2) to determine the effect of distributing a four hour exposure over four one hour periods separated by various intervals. These studies are in progress. At the present we have determined that the threshold of damage at 514.5 nm with an exposure duration of 4 hour, 1 hour, or 0.25 hour is 10,20 and 90 mW/cm^2, respectively (Figures 2,3,4).

To determine the effect of repeated exposures we began a series of experiments in which a total exposure of 4 hour's duration was divided into 4 one hour exposures. The set of

	d(cm)	a(cm)	S(cm^2)
Rhesus	1.9	1.6	2.54
Cynomolgus	1.8	1.0	0.89

d - diameter of globe
a - diameter of circle described by intersection of illuminating cone of light with posterior pole

$$S = 2\pi \frac{d^2}{4} \{1-\cos(\arcsin \frac{a}{d})\}$$

Table 1. Average Dimension of Globe

DURATION (hr)

λ (nm)	4	1	0.25
457.9	8	4	-
514.5	25	13	12
590	13	-	-
488	11	-	-
476	4	-	-

Table IIa. Matrix showing number of eyes exposed in single session under conditions given. Cells above the dashed line will be completed first. N>13/cell is required to adequately define the damage vs. intensity relationship.

INTERVAL (da)

λ (nm)	1	3	7
457.9	-	-	2
514.5	9	2	3
590	-	-	-
488	-	-	2
476	-	1	2

Table IIb. Matrix showing number eyes exposed in four, one-hour sessions under the conditions indicated. Cells above the dashed line will be completed first. N>13/cell is required to adequately define the damage vs. intensity relationship.

FIGURE 2

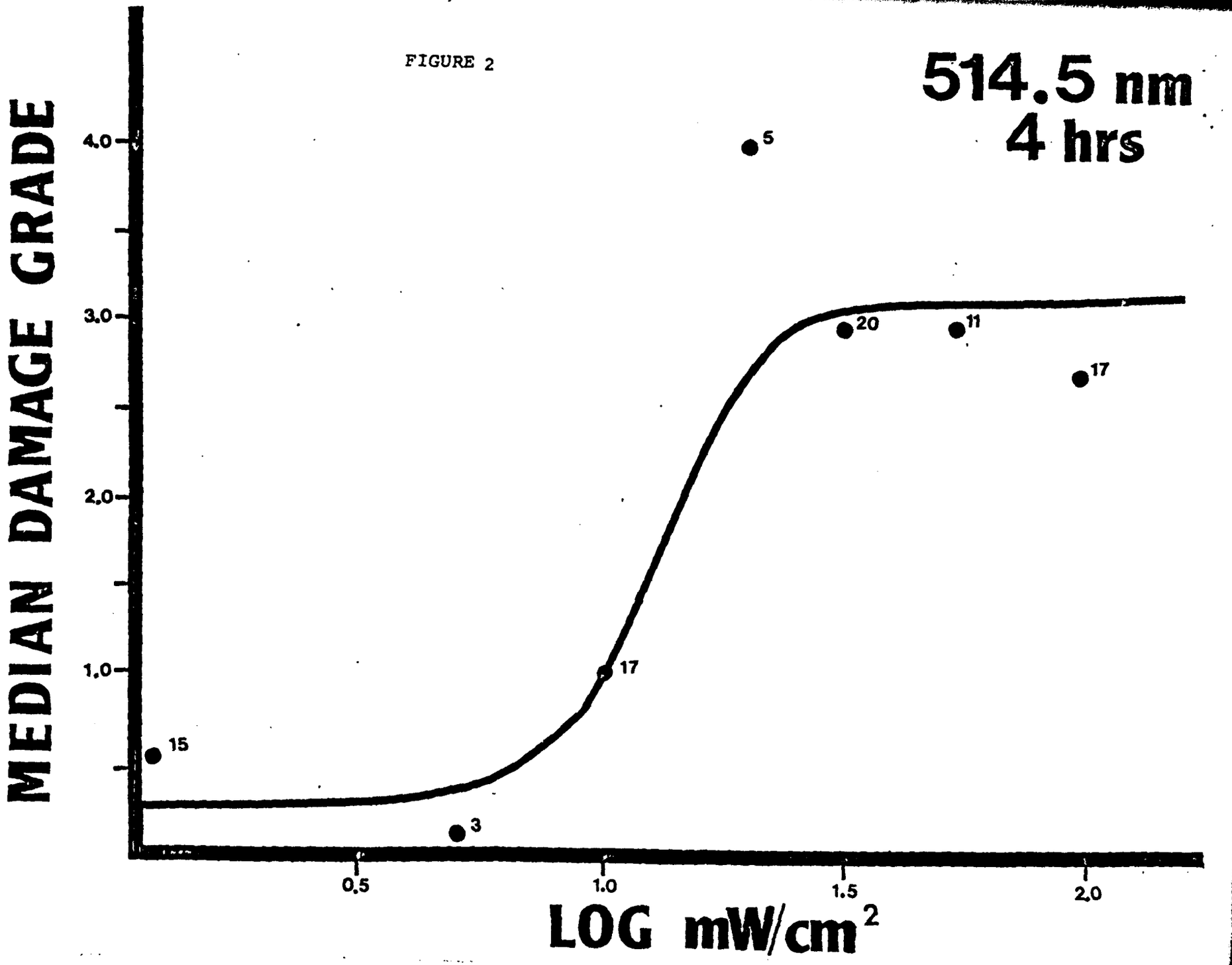

514.5 nm
4 hrs
MEDIAN DAMAGE GRADE
4.0
3.0
2.0
1.0
5
20
11
17
17
15
3
0.5
1.0
1.5
2.0
LOG mW/cm²

FIGURE 3

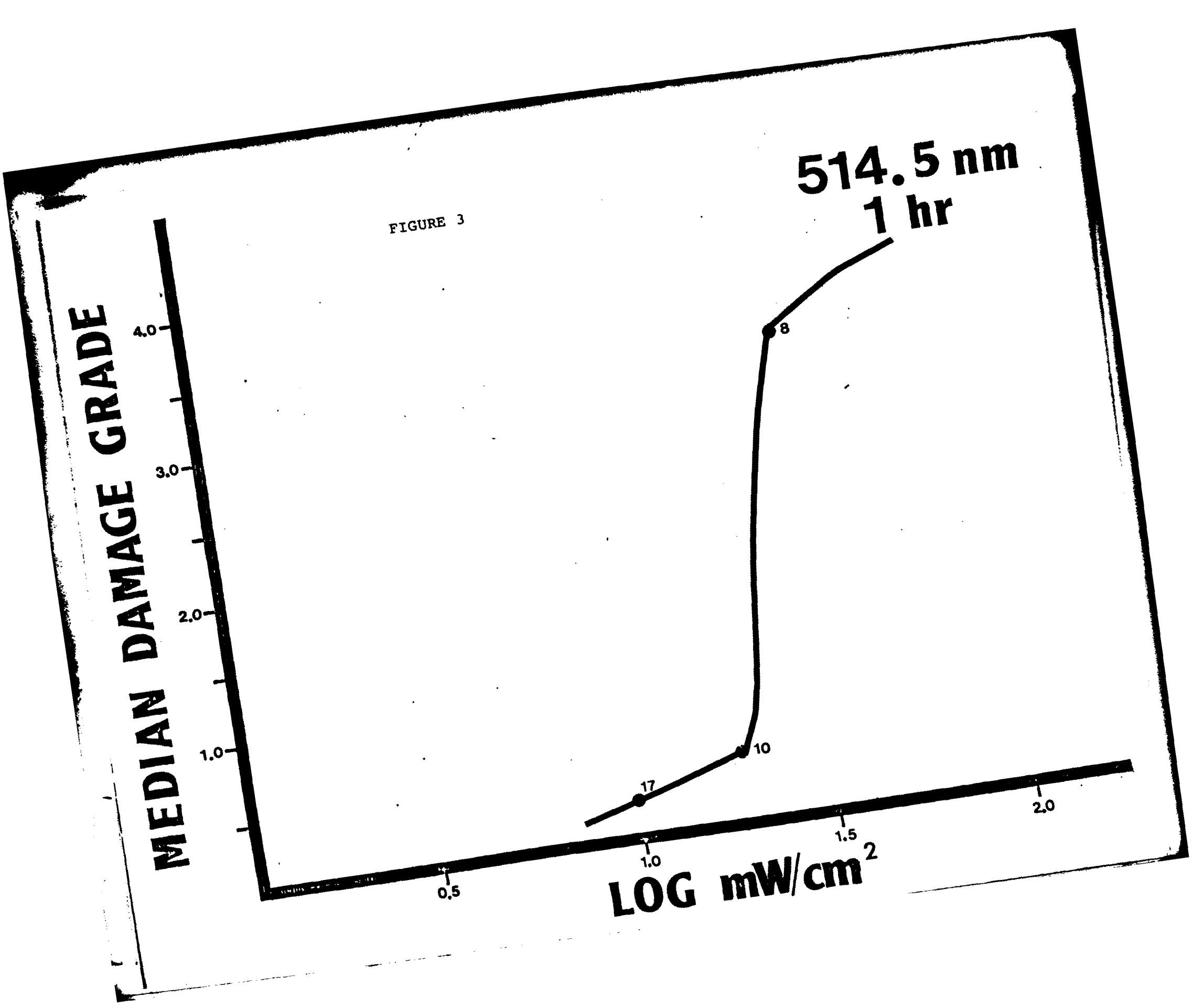
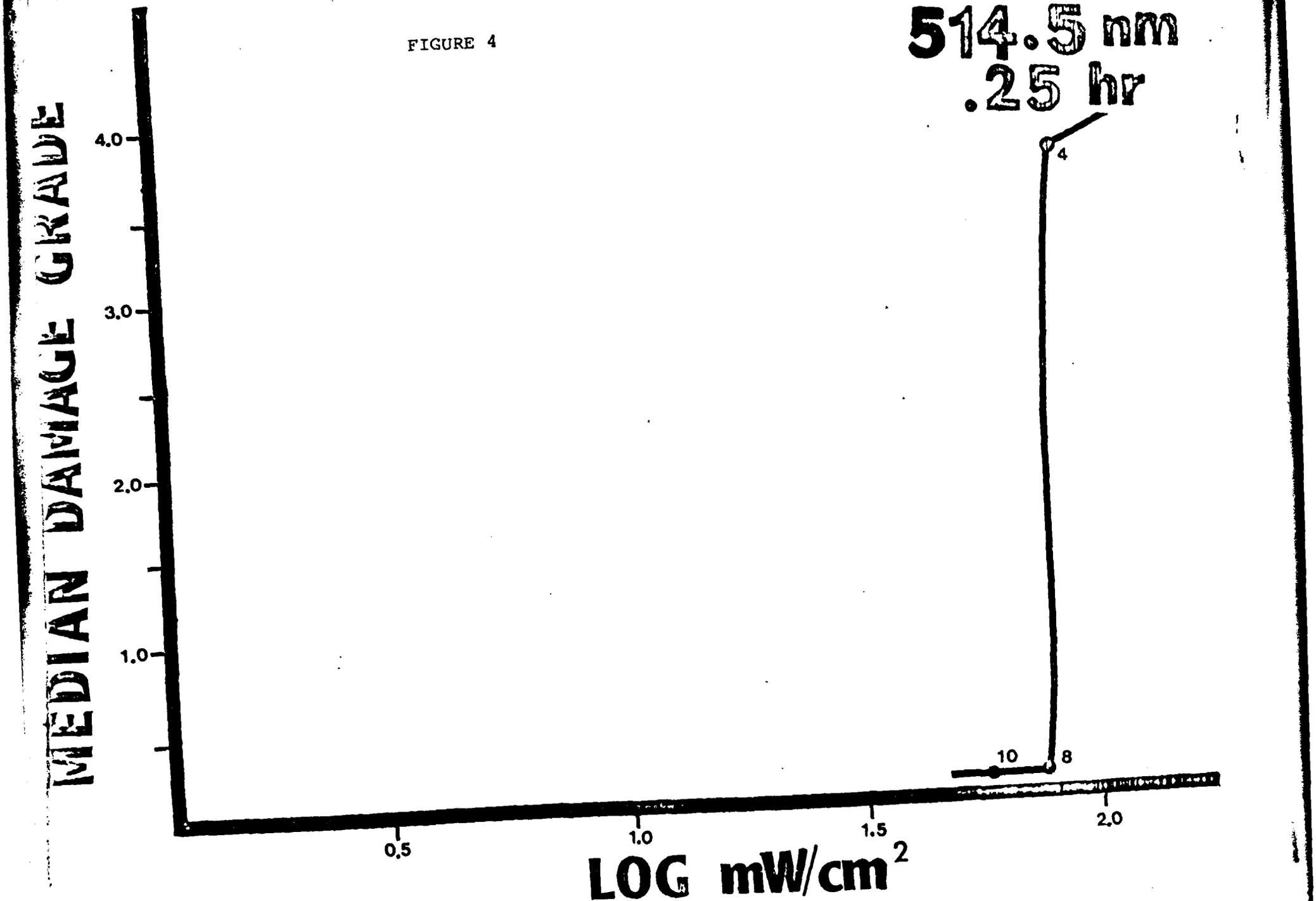

514.5 nm
1 hr
MEDIAN DAMAGE GRADE
4.0
3.0
2.0
1.0
8
10
17
0.5
1.0
1.5
2.0
LOG mW/cm^2

FIGURE 4


514.5 nm
.25 hr
MEDIAN DAMAGE GRADE
4.0
3.0
2.0
1.0
4
10
8
0.5
1.0
1.5
2.0
LOG mW/cm2

intervals between exposures chosen was 1 day or 7 days. The respective damage thresholds at 24 hour intervals was found to be 10mW/cm^2. Data is still being taken to complete the threshold determination at 7 day intervals but it appears to fall in the neighborhood of 20-30mW/cm^2. It was the preliminary determination of this threshold at 20-30mW/cm^2 level that led us to interrupt the 7 day series and work on the single one hour threshold. Since the two are so similar, there appears to be little additivity of effort when near threshold exposures of one hour's duration are repeated at weekly intervals.

As part of our objective to determine the mechanism of light induced retinal damage we have undertaken to measure the pattern evoked spectral electroretinogram. The post-exposure spectral ERG has been measured following exposures at 457.9 (Fig. 5a,b) and 514.5nm (Fig. 6a,b) at near threshold levels. Significant shifts in spectral sensitivity of the ERG in the range of 460 to 520nm have been observed. We are in the process of including 590nm exposures in this series to determine if differential cone damage is produced.

To extend our battery of functional measure of the damaging effects of light we have modified our pattern ERG procedure to allow us to measure pattern contrast sensitivity of the visual evoked reponse (VER) in exposed monkeys. To date we have tested the apparatus and procedures on other less expensive, cat, (Fig. 7a) and more cooperative, human (Fig. 7b), species. These data demonstrate that with this technique we can provide a measure of visual function which is highly correlated with acuity. On human subjects tested, extrapolation of the descending, high spatial frequency limb of the VER amplitude vs pattern

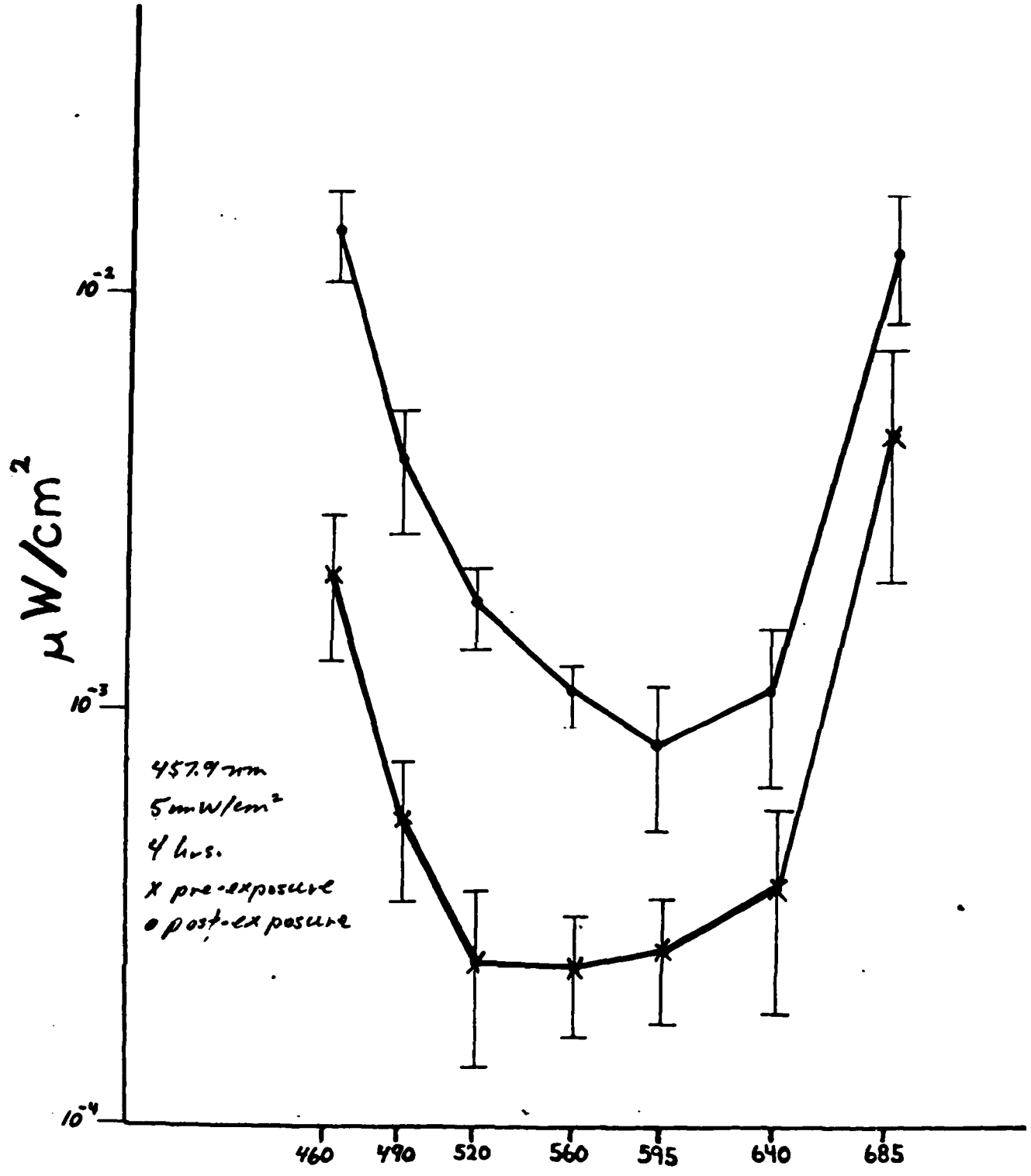

10^{-2}
10^{-3}
10^{-4}
$\mu W/cm^2$
457.9 nm
5 mW/cm²
4 hrs.
x pre-exposure
o post-exposure
460
490
520
560
595
640
685
nm

Figure 5a

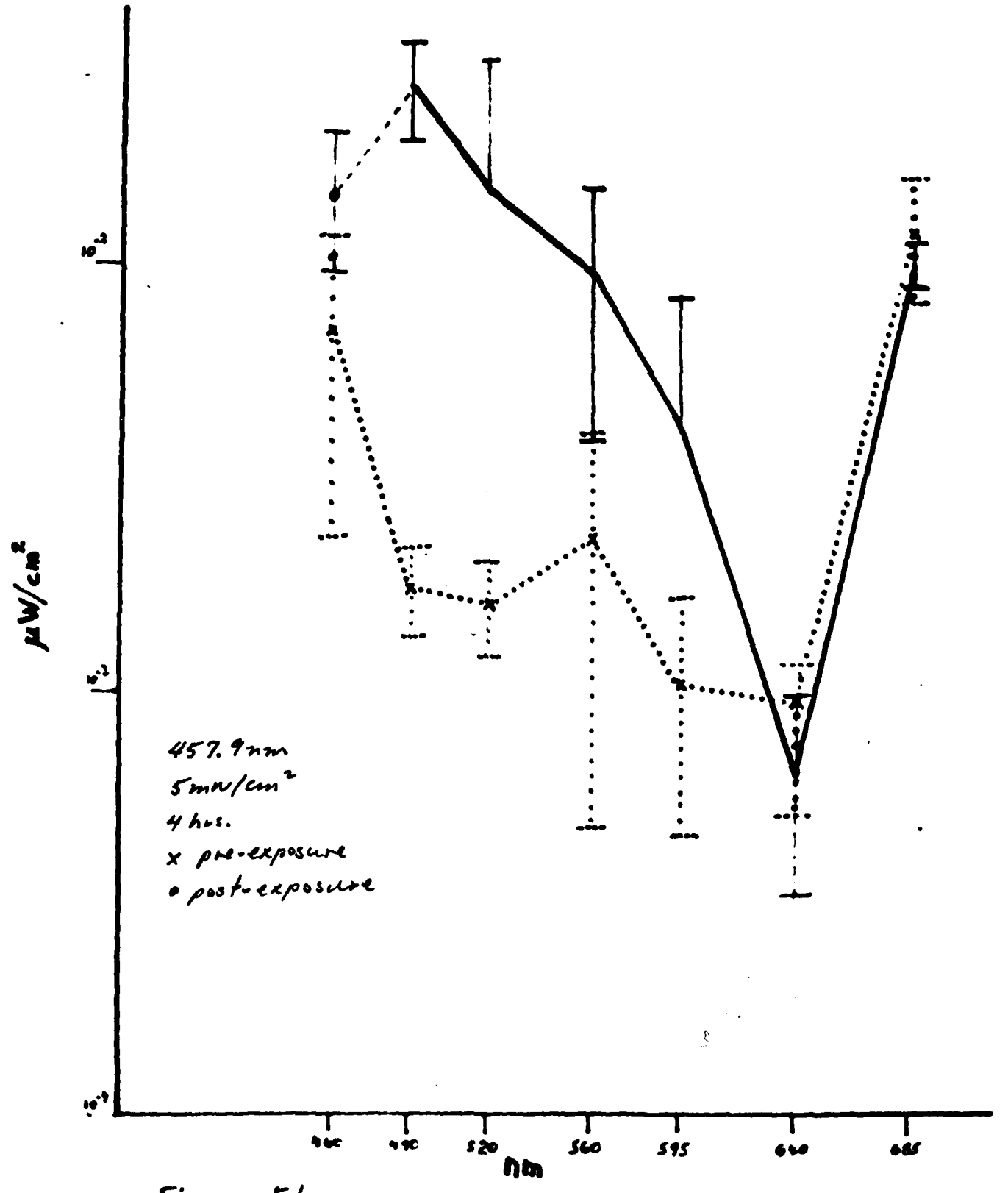


Figure 5b

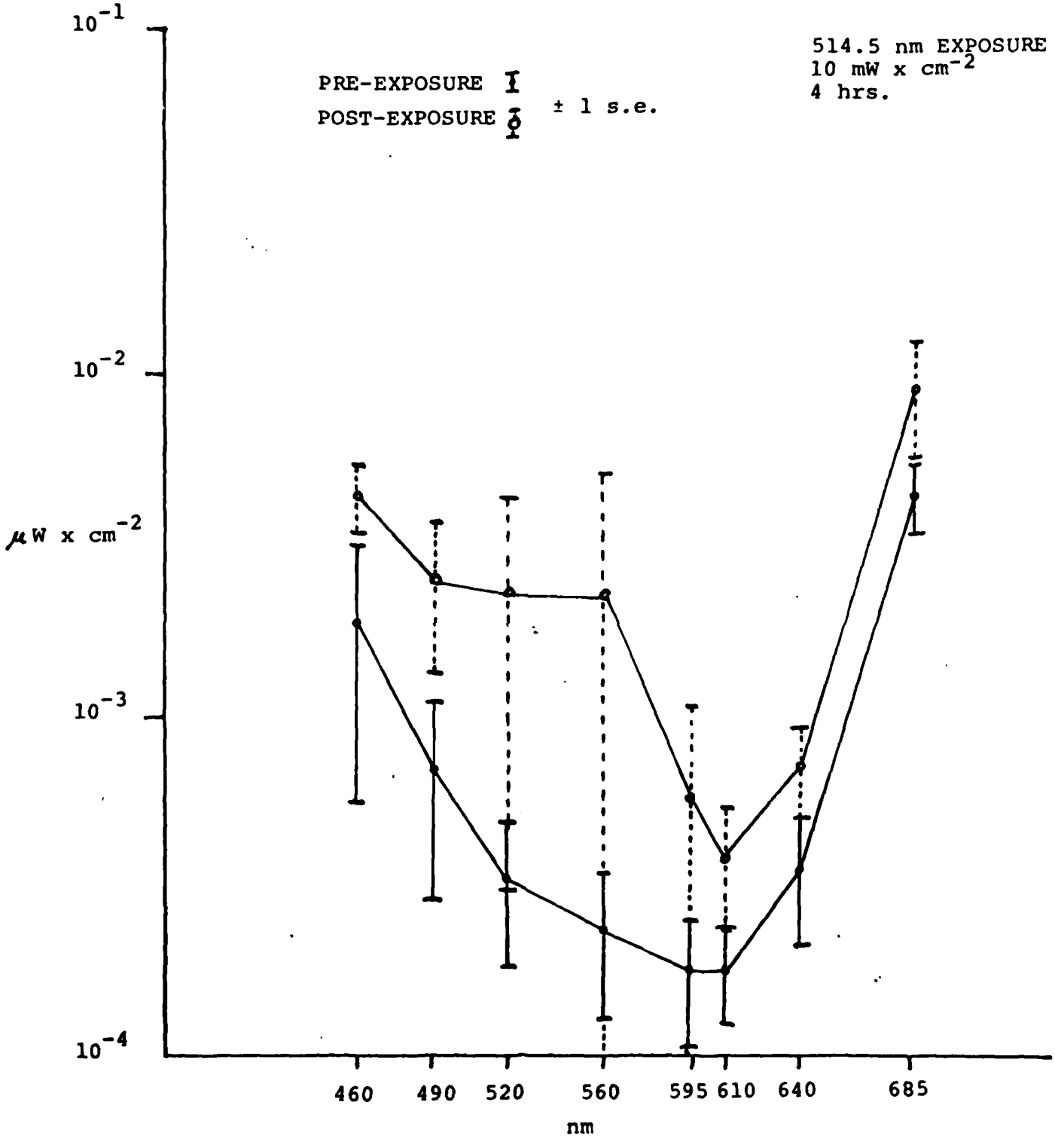

514.5 nm EXPOSURE
10 mW x cm^{-2}
4 hrs.
PRE-EXPOSURE
POST-EXPOSURE
± 1 s.e.
10^{-1}
10^{-2}
10^{-3}
10^{-4}
μW x cm^{-2}
460
490
520
560
595
610
640
685
nm

FIGURE 6a

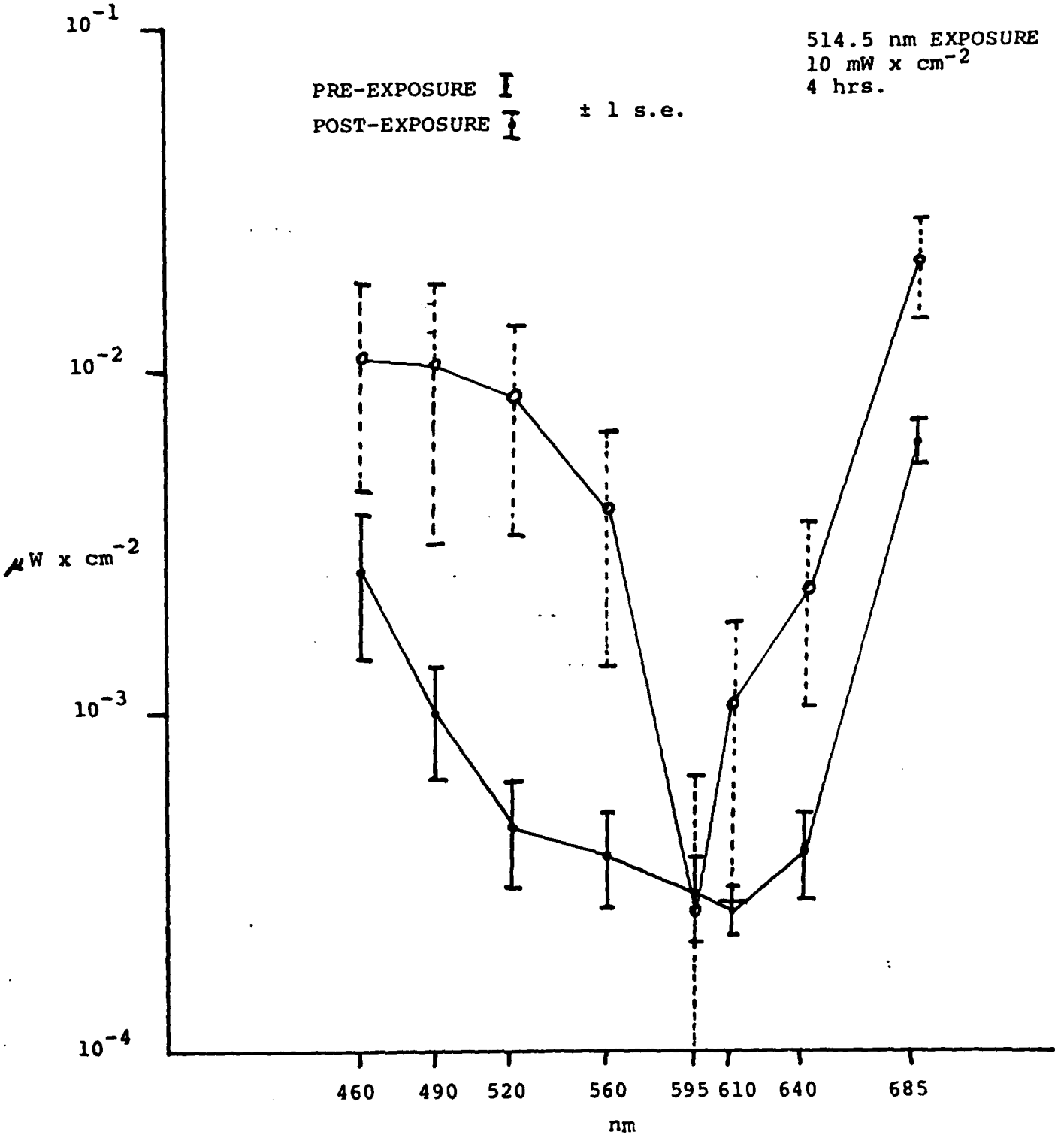

514.5 nm EXPOSURE
10 mW x cm-2
4 hrs.
PRE-EXPOSURE
POST-EXPOSURE
± 1 s.e.
10-1
10-2
10-3
10-4
μW x cm-2
460
490
520
560
595
610
640
685
nm

FIGURE 6b

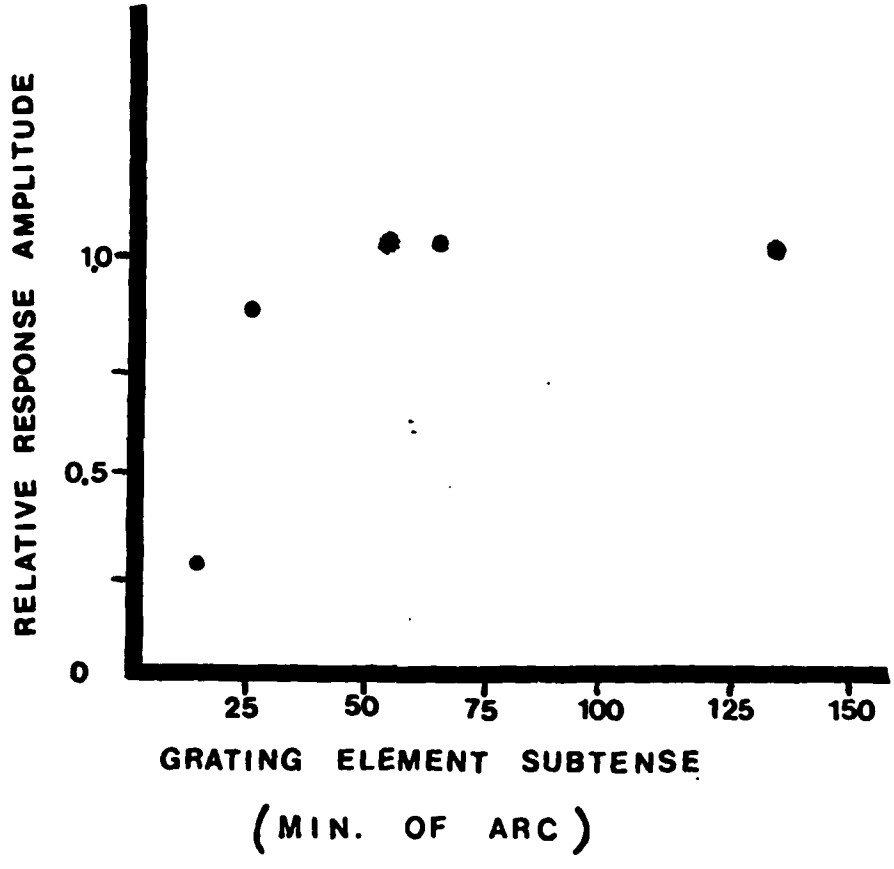


Fig. 7a This figure shows the relationship between visual evoked response amplitude and grating period for the cat. The VER was recorded from epidural stainless steel screws implanted in the skull. Extrapolation of the curve to zero amplitude corresponds to a grating spatial frequency of 4 cycles per degree.

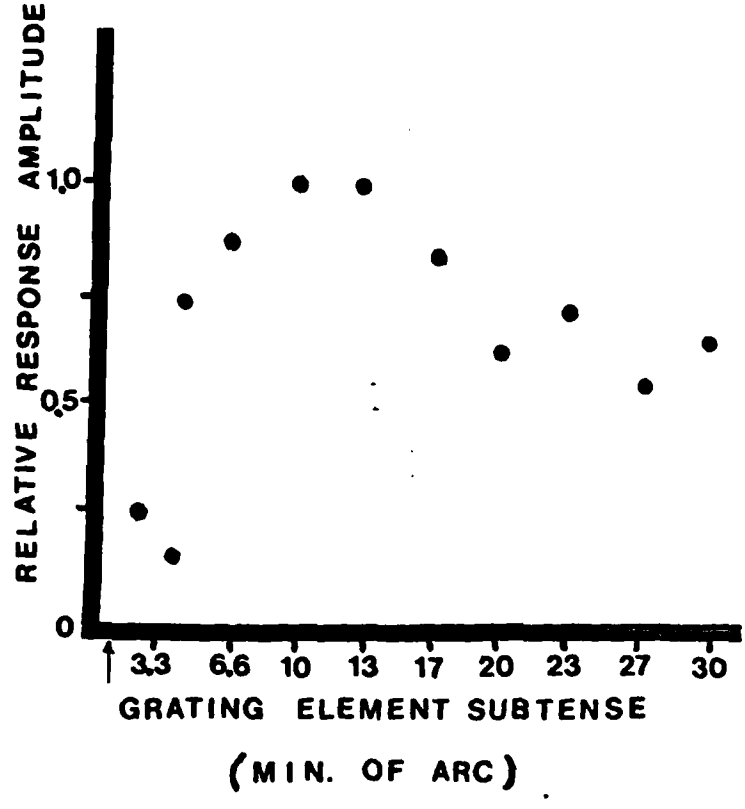


Fig. 7b Visual evoked responses in the human were elicited by gratings of various spatial periods. The electrodes were platinum needles imbedded in the epidermis of the scalp. Extrapolation of the function to zero amplitude corresponds to a spatial frequency threshold which was at the psychophysical detection for that subject.

element size to zero amplitude intercepts the abscissa at the psychophysical threshold for pattern detection in the test apparatus.

We have been able to successfully culture bovine pigmented retinal epithelium. Threshold data have been obtained for two lines of the argon laser. The criterion for damage is cell death as determined by histologic study of the stained culture plate. The thresholds at 514.5 and 457.9 nm are both less than 50 mW/cm^2.

Discussion

This line of research has led to several interesting and unexpected conclusions. When this work was first begun, the available evidence in the literature suggested that the damaging effects of light on the retina were mediated by a visual pigment and that the primary site of effect was the photoreceptor outer segment and pigment epithelium. We soon determined that the action spectrum of damage did not fit a visual pigment absorption curve. The threshold for damage at 457.9 nm was one log unit below that at 514.5 nm. We also found that while the cells most consistently affected were photoreceptors and pigment epithelium other retinal cell types were also damaged. In the range within one log unit above damage threshold all cellular elements were susceptible to being damaged.

The shape of the irradiance-duration reciprocity function was unexpected. The 0.25cm (900 sec.) threshold at 90 mW/cm^2 is only eight times the 4 hour threshold rather than 16 times as the reciprocity hypothesis would predict. However, the 0.25 hr threshold is approximately 4.5 times the one hour threshold. Our conclusion is that beyond one hour of exposure little additional damage is produced. This conclusion is supported by the observation that the damage threshold for 4 distributed one hour exposures is 8 mW/cm^2 at 24 hour intervals and 20-30 mW/cm^2 at 7 day intervals. Given the levels of irradiance required to produce just detectable amounts of damage, with our evaluation procedure, it appears that the insult occurs by the first hour with a threshold of from 10-30 mW/cm^2. Additional exposure produce

little additional effect. These data complement the findings of Ham, et al, (1979) which show reciprocity for 441.6 and 514.5 nm exposures from 10 to 1000 second duration. Our results extend their relationship to 3600 seconds; but beyond that period proportionally more energy (Joules) is required for exposures longer than 3600 seconds. Our thresholds for comparable exposure conditions are lower than Ham et al, 1979) by about 0.2 log unit. However, their criteria for damage is a funduscopically visible lesion. Our threshold determination reflects a contribution from functional and histopathological measures and should be slightly more sensitive.

Since the action spectrum of damage in our model doesn't correlate with the absorption spectrum of the visual pigment, what is absorbing the light? Some investigators (Ham, et al, 1979) have noted a rough correspondence between the action spectrum of damage and the absorption spectrum of melanin as well as the involvement of the retinal pigment epithelium at low levels of exposure. A melanin hypothesis is difficult to support however. While the role of species pigmentation on light damage is unsettled (Reuter and Hobbelen, 1977; Rapp and William, 1980; Howell and William, 1980) there is no evidence to show that the albino is less sensitive to light damage than is a pigmented species. We have noted in our rabbit studies that in animals with varigated pigmentation of the fundus, the pattern of damage is not correlated with pattern of pigmentation. In addition, we have noted in monkeys exposed to near threshold levels of light that there are areas of retina in which the retinal pigment epithelium appears normal in the face of disrupted and disorganized outer segments.

disrupted and disorganized outer segments.

We will address the pigmentation issue directly in the near future utilizing our bovine retinal pigment epithelium tissue culture model. We have been successful in differentially harvesting pigmented and non pigmented retinal epithelium from the cow eye. Pigmented cells lie outside while non pigmented cells lie over the tapetal area of the bovine fundus. We will determine the threshold for damage in each of these cell types. Assuming we find no other significant difference between these types other than pigmentation, the study should be decisive in evaluating the merit of the melanin hypothesis. The validity of the bovine retinal pigment epithelium culture model is presently under study. The threshold of damage as crudely measured by cell death is in the range of irradiance effective in vivo. Whether the type of effect of the cellular level is similar has not been determined.

We have adopted several strategies to reveal the involvement of other chromophores in the light damage process. Other researchers (Harwerth and Sperling, 1975) have noted selective receptor damage in the monkey retina. While our histological results have not shown that such changes occur, we are continuing to develop other more sensitive tests of these effects. The use of the pattern evoked spectral electroretinogram will provide this kind of information. Our results to date suggest that a differential cone effect does occur. However, we have analyzed the dates from only 457.9 and 514.5 nm exposures. It is necessary to expose of 590 nm to confirm the hypothesis of selective cone damage. If we find a reduced spectral sensitivity in the same spectral range as the exposing

wavelength, this would provide strong support that sub-population of cones are being affected.

We have begun a project, partially funded from other sources, which will allow us to measure in vivo, visual pigment regeneration kinetics biochemical constituents following light exposure. A fundus reflectometer with digital photon-counting system is under construction. It will be combined with the exposure optics to make spectral reflectance measures during and after exposure. These results will provide information on the metabolism of photochemicals in the retina; and if a normal chromophore is produced as a by-product of exposure and is present in sufficient quantity, we should be able to detect it.

Summary

Our research in light damage is evolving. Emphasis has been placed on careful measurement of the physical variables, parametric manipulation of these variables and a multifactorial asessment of the effects produced. This constitutes what could be termed a descriptive stage of research. This information is essential to the classification and organization of information. It gives us an answer to the question of "what" it is we are studying. These data are essential in planning the next phase of investigation in which the emphasis will be to explain the phenomenon of light damage. In this phase we will determine what biochemical entity is absorbing the light energy and the mechanism by which cellular function is altered leading to a pathological state. With an understanding of this process, investigation will move into the final phase of determining

a means of manipulating or controlling the system to whatever advantage we choose.

Recommendations

In light of the findings of this study, I would recommend continued investigation into the chronic effects of light on the retina - thresholds and mechanisms. The importance to the military is great because those levels of light which cause damage to the retina are close to everyday environmental levels, and in the military situation uncomfortably high levels might be endured to complete a mission when the conditions would not be endured in the normal civilian situation.

Some areas which need further examination: (1) the increased sensitivity at the blue end of the spectrum; (2) the cumulative effect of repeated exposures; and (3) the mixture of mechanisms which control the several types of damage in different animals and which have different action spectra as well as thresholds.

BIBLIOGRAPHY

Clarke, A. M., Geeraets, W. J. and Ham, W. T., Jr. An equilibrium thermal model for retinal injury from optical sources. Applied Optics 8: 1051-1054, 1969.

Gonasun, L. M. and Potts, A. In vitro inhibition of protein synthesis in the retinal pigment epithelium by chloroquine. Invest. Ophthalmol. 13 (2): 107, 1974. Also unpublished results by Gonasun and Potts.

Ham, W. T., Jr., Mueller, H. A., Ruffalo, J. J., Jr. and Clarke, A. M. Sensitivity of the retina to radiation damage as a function of wavelength. Photochemistry and Photobiology 29: 735-743, 1979.

Ham, W. T., Jr., Mueller, H. A., and Sliny, D. H. Thermal vs. photochemical retinal radiation damage. Nature 260: 153-155, 1976.

Harwerth, R. S. and Sperling, H. G. Effects of intense visible radiation on the increment-threshold spectral sensitivity of the rhesus monkey eye. Vision Res. 15: 1193-1204, 1975.

Howell, W. L. and Williams, T. P. Melanosomes of the retinal pigment epithelium: Distribution and correlation with light-damage. Supplement to Invest. Ophthalmol. and Visual Science 15: 190, April 1980.

Lawwill, T. The ERG and its correlation with damage caused by chronic exposure to light. Documenta Ophthalmologica Proceedings Series 2: 65-76, August 1972, Xth ISCERG Symposium, Los Angeles. Jerome T. Pearlman (editor).

Lawwill, T. Effects of prolonged exposure of rabbit retina to low-intensity light. Invest. Ophthalmol. 12: 45-51, 1973a.

Lawwill, T., Sharp, F. and Speed, N. Study of ocular effects of chronic exposure to laser radiation. Report #III, Army Contract DADA17-68-8105, 1973b.

Lawwill, T., Crockett, S. and Currier, G. J. Mechanisms of retinal damage from chronic laser radiation. Report #II, Army Contract DAMD17-74-C-4026, 1976.

Lawwill, T., Crockett, S. and Currier, G. J. Functional and histological measures of retinal damage in chronic light exposure. Documenta Ophthalmologica Proceedings Series 15: 285-295, 1977, XVth ISCERG symposium, Ghent. Jules Francois and Alfred De Rouck (editors), June 1977a.

Lawwill, T., Crockett, S. and Currier, G. J. Retinal damage secondary to chronic light exposure, thresholds and mechanisms. Documenta Ophthalmologica 44 (2): 379-402, 1977b.

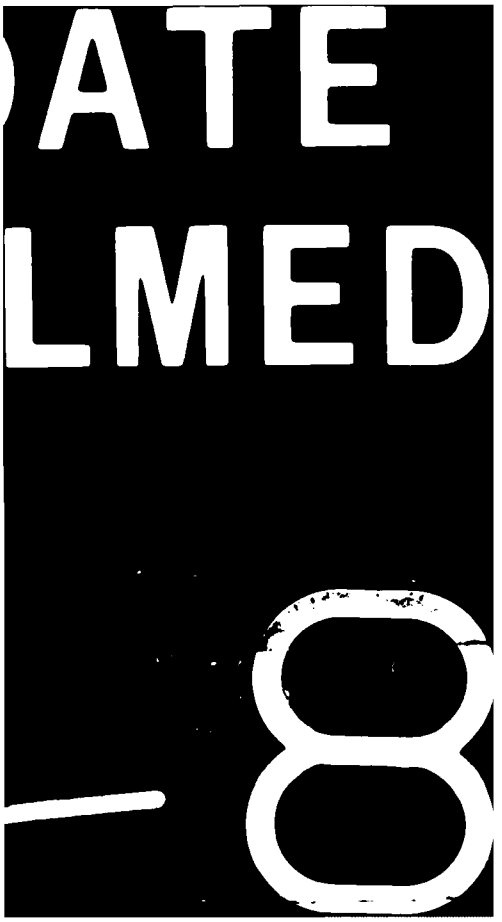
Noell, W. K., Walker, V. D., Kand, B. S. and Berman, S. Retinal damage by light in rats. Invest. Ophthalmol. 5: 450-473, 1966.

Noell, W. K. and Albrecht, R. Irreversible effects of visible light on the retina, role of vitamin A. Science 172: 76-80, April 1971.

Rapp, L. M. and Williams, T. P. The role of ocular pigmentation in protecting against light-induced damage to the rat retina. Supplement to Invest. Ophthalmol. and Visual Science 189: April 1980.

Reuter, J. H. and Hobbelen, J. F. The effect of continuous light exposure on the retina in albino and pigmented rats. Physiology and Behavior 18: 939-944, 1977.

Ripps, H. and Snapper, A. G. Computer analysis of photochemical changes in the human retina. Computer in Medicine and Biology 4: 107-122, 1974.


ATE
LMED
8